Partial Purification of Sea Nettle *(Chrysaora quinquecirrha)* Nematocyst Toxin[1] (34608)

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(Introduced by Frank H. J. Figge)

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Chrysaora quinquecirrha, the sea nettle, is capable of inflicting painful cutaneous lesions upon bathers who come in contact with its tentacles. Injections of toxin from the jellyfish nematocyst suspensions are lethal to mice and produce cardiac changes in rats, but the pathogenesis of these reactions is not completely understood (1, 2). Some chemical and physical properties of nettle nematocyst suspensions and their toxin were investigated in order to further define the pathophysiology of the sting.

Materials and Methods. Fresh *Chrysaora* tentacles (WT) were obtained by previously described techniques (1). All chemical reagents and gels were obtained commercially.

Protein analyses were performed according to the technique of Waddell (3). For purposes of comparing the present data with previously reported results, the protein concentrations were divided by 6.25 to convert them to nitrogen concentrations.

Total LD_{50}'s were calculated in terms of N_2 content using 25 g as the average weight of a mouse. The lethal potency or specific activity was expressed as μg N_2/g mouse present in the 50% lethal intraperitoneal dose (LD_{50}).

Nonspecific esterases and hyaluronidase concentrations were determined colorimetrically (4, 5). Human erythrocyte 2-hr glucose uptake was investigated before and after the introduction of toxin into the system. The incubation was performed at 37° in an atmosphere of 5% CO_2. Glucose levels were analyzed according to a standard technique (6).

Sonic treatment, disc electrophoresis, and the measurements of dermal necrosis and human erythrocyte hemolysin were conducted according to previously described methods (7–10).

Results. Homogenization of WT in a blender for 5 sec was insufficient to break up the tentacle cells. No intact cells could be seen by light microscopy in tentacles which had been subjected to 15–30 sec of blending. The nematocysts were collected by centrifugation at different speeds (1,000–10,000*g*) for 5–40 min. A centrifugal force of at least 5000*g* applied for 10 min was required to produce a clear supernatant fluid.

It was hoped that most of the toxin would be present in the sediment which contained the nematocysts. Unfortunately, the bulk of the toxin was always in the supernatant fluid regardless of the speed or duration of the centrifugation. The addition of sucrose (final concentration=6%) to the WT before homogenization did not decrease the number of ruptured nematocysts. The toxicity in the sediment was also unaffected by the presence of sucrose.

Active toxin was released from the organelles by grinding the nematocyst suspension (NS) with a precooled (—60°) mortar and pestle in an ice bath. In this manner the toxicity of NS could be increased approximately 50%, providing the grinding procedure was continued for 20–45 min. The addition of sand or alumina to the mortar did not increase the efficiency of grinding. Sonic treatment of NS with 1 A for at least 90 – 150 sec ruptured the nematocyst and released

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an amount of toxin equal to that detected in ground NS.

A slight purification of the toxic principle was accomplished by ultracentrifugation (40,000–100,000g) of the ground NS for 1 hr. The aqueous supernatant fluid SU contained more toxicity (total LD_{50} units) and a lower or more potent specific activity (μg N_2/g mouse) than the sediment.

Grinding NS with 8 M urea or dimethylsulfoxide (DMSO) destroyed 50% and 25% of its lethal activity, respectively. The incorporation of 6.15 M 2 mercaptoethanol (2 ME) and 2.5 $\times$ 10^{-3} M ethylenediaminetetraacetate (EDTA) in the grinding fluid resulted in 2- to 3-fold higher yields of total toxin. Simultaneous inoculation of 2 ME–EDTA solution and ground NS into the animal at separate injection sites was not more lethal than injection of ground NS alone. The use of 2 ME and EDTA was subsequently discarded because they produced interference with the protein determinations.

The toxicity of the NS was unaffected by freeze-drying. The lyophilized NS was stable at 25° for at least 48 hr. Most of the toxic activity (75%) of lyophilized NS could be destroyed by exposure to acetone, ether, or alcohol at 4° for 10 min. The lethal activity of NS was found to be stable for at least 6 months at —60° but a 50% loss of toxicity resulted from storage at —10° for 5 days and a 90% loss after exposure to 56° heat for 15 min. No loss in SU potency was observed

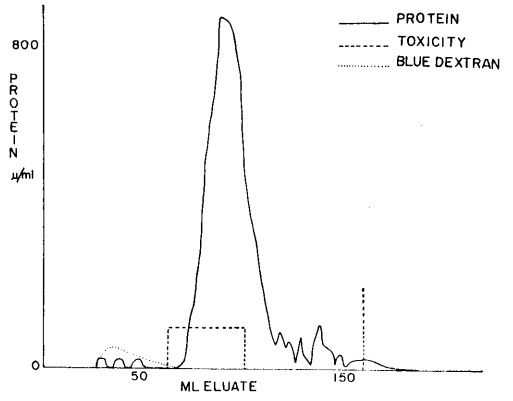

FIG. 1. Passage of nematocyst toxin through a Sephadex G-200 column.

TABLE I. Potency of Various Nematocyst Preparations during Partial Purification.

	Specific activity of preparation (μg N_2/g mouse)
Whole tentacle (WT) (range)	3.70–6.30
Nematocyst suspension (NS) (average)	3.50
Supernatant fluid after grinding and ultracentrifugation (SU)	1.75
Pool of toxin after passage through Sephadex G-200	
Initial fraction	0.98
Second fraction	0.32

after 1 hr of treatment with different phosphate buffers (pH 6–9.5) which contained variable amounts of saline (0.001–0.3 M). Overnight exposure of the SU to 0.001 M phosphate buffer (pH 4) at 4° resulted in 50% loss of potency. The SU activity was unaffected by similar treatment with 0.001 M phosphate buffer (pH 9.5). Three successive cycles of freeze (—60°) thaw did not destroy the toxic principle. When compared with an appropriate control, more than 88% of the potency was lost after treatment of SU with 0.2% trypsin (pH 7.0 with an incubation period of 1 hr at 25° followed by 2 hr at 4°).

Dialysis of SU against large quantities of water produced a variable loss in toxic activity (30–85%). The loss of toxin was always less than that expected by diffusion to a larger fluid compartment through the membrane bag. The toxic component could not be recovered after lyophilization of the fluids inside or outside the dialysis bag.

The toxic component of SU was able to pass a 400-mμ Millipore filter and was not altered when placed inside a membrane filter (11).

The SU toxin was excluded from the interstices of Sephadex G-25 columns. Passage of the toxin through columns (30-cm height $\times$ 2-cm diameter) was delayed by Sephadex G-200 gel (Fig. 1). In these experiments most lethal activity came off the column in those fractions which contained the ascending portion of the major protein curve. A significant amount of lethal activity came of Sephadex G-200, 100, and 75 columns after the major

protein curve. More than 90% of the toxic activity was recovered, and partial purification could be obtained by passage through the Sephadex G-200 columns (Table I).

The initial lethal fraction possessed hemolytic and dermonecrotic properties as well as a cardiotoxic action (2). The later, smaller, potent, lethal fraction was located within a wider zone which had dermonecrotic activity but no hemolysin.

The initial toxic fraction recovered from the G-200 gel was lyophilized. Disc electrophoresis performed on the material revealed multiple protein bands. Toxic activity was recovered only from the first centimeter of the spacer gel.

Several enzymatic studies were done on the SU in an attempt to find a rapid *in vitro* test which would correlate with the mouse lethal factors. Nonspecific esterases were present in the SU but they did not correlate with the lethal activity. No hyaluronidase could be detected in an inocula of SU containing 3 LD_{50} doses. Two-hour glucose uptake of human erythrocytes was not impaired after inoculation of NS having 5 LD_{50} doses.

Discussion. In our experiments homogenization of the tentacle is performed in order to isolate the nematocyst, the organelle responsible for this animal's sting. The homogenization should be efficient enough to rupture the tentacle cells while being gentle enough to leave the nematocyst undischarged. Centrifugation of the homogenized tentacles should sediment the nematocysts but not allow the toxin to denature or diffuse out of the organelle. Unfortunately, this was not accomplished under the above conditions. Freeman and Turner had similar problems with *Chironex* nematocysts (12). Since it is so difficult to physically treat the tentacles without discharging the nematocyst or releasing the toxin, a technique for preparing NS was arbitrarily defined as: (1) blending for 30 sec; (2) centrifugation (2000*g*) for 10 min; (3) washing 1 × with 3 vol of filtered bay water; and (4) repeat centrifugation. The washed nematocyst sediment was stored at −60° until used. The toxicity of WT was variable. The LD_{50} value detected in these experiments (Table I) was significantly lower than that reported earlier (1). There is no current explanation for this observation.

Release of sea nettle toxin from its nematocysts appears to be best accomplished by gentle grinding or sonic treatment. Previous experiments revealed that more strenuous physical treatments of nematocyst suspensions resulted in an aqueous supernatant fluid which was less potent than the sedimented nematocyst particles (1). Rapid sonic treatment must be performed at temperatures below 25° in order to prevent denaturation.

Lyophilization did not affect the toxin in the present experiments. A previous paper reported loss of toxicity with this procedure (1). This discrepancy is best explained by the fact that better care was taken to insure rapid freezing and complete lyophilization during the above experiments.

The increased potency of ground or sonicated suspensions indicates that mice tolerate an injection of toxin containing intact nematocysts better than one with ruptured organelles. After injection, toxin within the nematocyst might be denatured before being released. Gradual rupture of the nematocysts might also result in a lower peak blood level of toxin.

Grinding in 2 ME and EDTA solutions yielded soluble toxin with even higher potency. The experiments in which the animal received separate injections of the 2 ME–EDTA solution and aqueous toxin indicate that these chemicals probably promoted more efficient nematocyst rupture rather than injuring the injected animal themselves.

Grinding or sonic treatment and subsequent ultracentrifugation produced a 2-fold purification step. The fact that the initial toxin entered the interstices of the G-200 and not the G-100 gel indicates that the molecular weight of the toxin or of a complex molecule containing the toxin is between 100,000 and 400,000. The molecular weight of the later lethal toxin is estimated at 5-70,000.

The toxin's trypsin lability indicates that a peptide split by trypsin is necessary for the

in vivo assay of NS. The loss of toxic potency after dialysis was significant but not great enough to be accounted for by dilution. In addition, reconstitution experiments did not recover the toxin. The gel filtration experiments showing that the toxin could only enter large pores suggest that diffusion across the membrane should not occur. It is possible that the membrane dialysis bag denatured the toxin.

SU appears to be stable in solutions having a pH of 6–9.0 containing 0.001–0.3 *M* NaCl. These experimental results indicate that further purification of the toxin on ion-exchange resins might be possible. Investigations along this line are now in progress.

Summary. Soluble toxin was released from fresh *Chrysaora quinquecirrha* (sea nettle) nematocysts by gentle grinding or sonic treatment in distilled water. The lethal activity was not decreased by three cycles of freezing and thawing nor by lyophilization. Organic solvents such as acetone, ether, alcohol, and dimethylsulfoxide inactivated the toxin. Passage of the toxin through Sephadex gel results in partial purification and separation of the lethal activity into at least 2 fractions which are presumably proteinaceous.

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